Research Paper | Botany | E-ISSN No : 2454-9916 | Volume : 3 | Issue : 9 | Sep 2017

ASSESSMENT OF ANTIFUNGAL ACTIVITY OF ONION (ALLIUM CEPA L.) BULB EXTRACTS

B. K. Singh

Department of Botany, R.H.S.P.G. College Singramau, Jaunpur-222175, U.P.

ABSTRACT

Onion (*Allium cepa* L.) is a well-known traditional nutraceutical and medicinal plant that is cultivated and used around the world. The present study was performed to evaluate the efficacy of different extracts of onion bulb on antifungal activity. The extracts were prepared with aqueous, ethanol, chloroform and petroleum ether and subjected for preliminary phytochemical and antifungal activity. Antifungal activity was assessed by the paper disc agar diffusion method. The results of the antifungal activity showed that the onion exhibited significant fungal growth inhibition in its aqueous and ethanolic extraction. Onion is most effective in its chloroform extract while its effectiveness is least in petroleum ether extract. Screening of antimicrobial activity performed on A. *cepa* bulb extracts shows that they are endowed with potentially exploitable antifungal activity. These results showed that onion bulb can be a source of compounds that can serve as templates for future fungicides against *Aspergillus niger*, *Aspergillus*, *fumigatus*, *Candida albicans* and *Aspergillus flavus*. Hence, bulb extracts of A. *cepa* could be used as an easy accessible source of antifungal agent making it one of the potent therapeutic phytomedicines.

KEYWORDS: Onion; Antifungal activity; Bulb extracts; Phytochemicals.

1. INTRODUCTION:

Onion (*Allium cepa* L.) is a multipurpose food plant that is used as traditional Indian spices. It has great health significance and is consumed for its putative nutritional and health benefits for centuries (Rose *et al*., 2005). Traditionally, onions and plants belonging to the *Allium* genus and *Allium* is the largest and important representative genus of the family *Liliaceae* which have been used as an herbal remedy for a wide range of ailments, due to their association with many pharmacological effects (Yin & Cheng, 1998; Rose *et al*., 2005). Biological effects attributed to onions have been commonly ascribed to the volatile sulfur-containing compounds, such as thiosulfinates, mainly responsible for the characteristic taste, aroma and lachrymatory effects (Lanzotti, 2006). However, these volatile products are highly unstable and recently attention has been focused on the effects of phenolic compounds, such as flavonoids, which are more stable (Ioku *et al*., 2001). Onion is known for being a good natural source of flavonoids mainly represented by the flavonols - quercetin and kaempferol, which are present as their glycosides (Fossen *et al*., 1998).

In recent years, many publications have reported evidence of beneficial health effects attributed to flavonoids including antiallergenic, antiinflammatory, cardioprotective, vasodilatory, anticarcenogenic and antioxidant properties (Shon *et al*., 2004). Several epidemiological studies have also associated the consumption of flavonoids with a reduction of the risk of chronic diseases including, cancer, diabetes and coronary heart problems (Hirvonen *et al*., 2001; Kosmider & Osiecka, 2004). The antibacterial and antifungal properties reported to be possessed by flavanoids has increased the interest of the food industry in these natural compounds as components to improve food stability against microbiological spoiling agents (Taguri *et al*., 2004; Sofia *et al*., 2007). Protection of food from pathogens and spoilage organisms has been traditionally achieved by chemical methods, but during recent years there has been an increase in consumer interest in developing foods which contain a low level or are free of chemical preservatives (Viuda-Martos *et al*., 2008). The emergence of pathogens which are resistant to classical preservatives has also created an urgent necessity to find alternative antimicrobial agents (Xu & Lee, 2001). In consequence, the food industry is interested in developing natural components for the total or partial replacement of synthetic antimicrobials (Grohs & Kunz, 2000).

Onions can be considered as a good source of natural additives to retard food deterioration (Navas *et al*., 2006). However, the application of thiosulfinates and volatile compounds for food preservation is limited due to their strong flavour and biochemical instability (Benkeblia, 2004). These properties focus attention on the more stable flavonoids as additives to enhance food shelf-life by inhibiting microbial spoiling and oxidative deterioration, due to their antimicrobial and antioxidant properties (Ramos *et al*., 2006; Naz *et al*., 2008). Therefore, this study intended to investigate the antifungal activity of onion bulb extract on selected fungi, to explore the preliminary phyto-chemical analysis, and to find the antibacterial properties of bulb extracts of *A. cepa* which are responsible for its pharmacological properties.

2. MATERIALS AND METHODS

2.1. Microorganisms:

Antifungal activities of aqueous, ethanol, chloroform and petroleum ether extracts of *A. cepa* were studied. Ketoconazole was used as standard drug. The microorganisms, maintained on Nutrient Agar (Merck, India), were supplied by the Laboratory of Mycopathology and Microbial Technology, Department of Botany, Banaras Hindu University, Varanasi. Four species of fungi, *Aspergillus niger, Aspergillus fumigatus, Candida albicans* and *Aspergillus flavus* were used in this study.

2.2. Collection and identification of plant material:

The plant material (onion) was selected for the investigation. Fresh onions were procured from the local market in Singramau, Jaunpur, Uttar Pradesh. The voucher specimens of the plants were authenticated by National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh and were sorted for uniformity and absence of defects and stored at 4°C prior to analysis.

2.3. Extract preparation of plant material:

2.3.1. Aqueous extract:

Fresh onion bulbs were peeled off their outer layer and 1 kg of onion bulbs was washed thoroughly with distilled water and then the bulb was cut into pieces and was made into a crude paste. This paste was soaked in 1 litre of sterile distilled water for 24 hours at 4°C and it was then filtered thrice using a sterile muslin cloth. The filtrate was poured into a beaker and concentrated on a water bath at 100°C to obtain semi-solid residue and aqueous extract was weighed and this was immediately subjected to antifungal analysis using standard method.

2.3.2. Ethanolic extract:

After cleaning 1kg of onion bulbs as described earlier they were made into a paste which was soaked in 500 ml of ethanol for 15 days at room temperature then it was filtered using sterile muslin cloth and the filtrate was poured into a beaker and concentrated on a water bath at 70-80°C to obtain semi-solid residue. The weight of the yield was noted and this was subjected to antifungal analysis.

2.3.3. Chloroform extract:

After making a paste of 1kg onion bulbs as described earlier, they were separately soaked in 300 ml of chloroform for a week at room temperature. It was then filtered using sterile muslin cloth and the filtrate was concentrated in a beaker at 60-62°C to obtain semi-solid residue. This was weighed and subjected to antifungal analysis**.**

2.3.4. Petroleum ether extract:

Following the earlier procedure, onion bulbs were prepared and soaked in 200 ml of petroleum ether for 15 days at room temperature. Then it was filtered and the filtrate was concentrated at 40-60°C. The extract was weighed and subjected to antifungal analysis**.**

2.4. Determination of antifungal activity:

The disc diffusion method was used (Gillespia, 2002) to evaluate the antifungal activity against *Aspergillus niger, Aspergillus fumigatus, Candida albicans* and *Aspergillus flavus.* The sterilized (autoclaved at 120°C for 30 minutes) medium was inoculated with the suspension of various microorganisms and poured into petridishes to give a depth of 5mm. Various extracts of *A. cepa* such as aqueous, ethanolic, chloroform and petroleum ether were prepared separately and concentration of each extract was maintained at 100µg/ml. Sterile disc (made from Whatman filter paper-41 was sterilized in UV lamp) dipped in specified concentration of the extracts and standard (ketoconazol, 50µg/ml). The impregnated discs are allowed to dry and dried discs were placed on the surface of agar plates.

A disc dipped in solution of different concentrations of *A. cepa* extracts and standard were placed on the surface of agar plates. The plates were left for 1hour at room temperature and incubated at 37°C for 24 hours. The diameter of zone of inhibition of extracts and standards were measured. All tests were performed in duplicate and antifungal activity was expressed as the mean diameter of inhibition zones (mm) produced by the formulated bulb extracts of *A. cepa*.

3. RESULTS AND DISCUSSION:

In recent years, a remarkable increase has been reported in the incidence of different mycoses due to aggressive cancer chemotherapy, widespread use of broad spectrum antibiotics, increasing number of immunosuppressive diseases and highly effective immunosupressants for organ transplantation (Anaissie *et al.,* 2003). Because of huge similarities between fungal and mammalian cells, there is a limited selective target for designing new antifungal formulations (Barrett, 2002). There is thus an urgent need for a new antifungal agent with new modes of action, broad fungicidal spectrum and fewer dose-limited side effects (Graybill, 1996). It has been reported that onion exerts a marked antifungal activity against both yeasts and mycelia fungi including dermatophytes (Barrett, 2002). To investigate, how different extracts of *A. cepa* act towards the important fungi, *Aspergillus niger, Aspergillus fumigatus, Candida albicans* and *Aspergillus flavus* were selected for the present study. Antifungal activities of aqueous, ethanolic, chloroform and petroleum ether extracts were shown (Table 1). The results reveal that the bulb of the onion has potential antifungal properties. The chloroform extract of onion showed highest zone of inhibition with *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans* but less in case of *Aspergillus flavus*. Petroleum ether extract of onion exerted least inhibition on *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans* and *Aspergillus flavus.* Aqueous and ethanolic extracts of onion showed significant inhibitory effects against *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans* and *Aspergillus flavus.* Finally, it is concluded that the onion exhibited significant fungal growth inhibition in its aqueous and ethanolic extraction. Onion is most effective in its chloroform extract while its effectiveness is least in petroleum ether extract.

Table 1: Antifungal activity of bulb extracts of *A. cepa* on selected fungi

Name of the fungi	Standard	Aqueous extract	Ethanolic extract	Chloroform extract	Petroleum ether extract
Aspergillus niger	37±1.5	21±0.8	23±1.1	28±1.4	12±0.7
Aspergillus fumigatus	37±1.5	20±0.7	22±0.8	31±1.3	11±0.8
Candida albicans	37±1.5	21±0.7	21±0.7	32±1.5	13±0.6
Aspergillus flavus	37±1.5	23±0.9	23±1.3	24±1.1	14±0.7

(*The values are diameter of zone of inhibition in mm at 100µg/ml concentration.*)

Regardless of antifungal activity of onion, it has been shown that crude extracts of onion may have potent antifungal and antibacterial properties (Elnima *et al.,* 1983). Phenolic compounds such as quercetin and kaempferol present in onion may contribute to this activity (Rauha *et al.,* 2000). In the present study also onion-induced dose-dependent fungal growth inhibition may be attributed to the presence of phenols and secondary metabolites. In accordance to these findings, reports by Skerget *et al.* (2005) can be quoted which recorded that the ethanol and acetone extracts of red onion's skin and edible part possess antifungal activity against *A. niger, T. viride* and *P. cyclopyum.* Moreover, it has been already reported that alliicin, thiosulfonates and other compounds of onion exhibit fungistatic activities against *A. niger, Rhodotorula nigricans, Penicillium italicum, Penicillium cyclopium, A. flavus, Cladosporium macrocarpum, A. fumigatus, A. alutaceus, A. terreus* and *Penicillium chryogenum* (Harris *et al.,* 2001). Finally, the onion-induced differential antifungal activities of *A. cepa* may be due to the presence of phenolic constituents recorded in the present study and it may also be due to the presence of other compounds such as allicin, thiosulfonates, etc (Harris *et al.,* 2001).

4. CONCLUSION:

In conclusion, the screening of antifungal activity performed on *A. cepa* bulb extracts, which is traditionally used as herbs, shows that they are endowed with potentially exploitable antifungal activity. Phytochemical constituents and total phenolic contents (quercetin and kaempferol) present in the onion could have contributed for the efficient inhibition of fungal growth. Hence, bulb extracts of *A. cepa* could be used as an easy accessible source of natural antifungal agent and therefore, onion bulb can be used as one of the effective therapeutic phytomedicines. However, additional purifications of the active compounds and *in vivo* evaluation of antioxidant and antifungal agents along with toxicity studies of the extracts from *A. cepa* are suggested for further studies.

Acknowledgements:
The author (BKS) is thankful to the Principal, R.H.S.P.G. College, Singramau, Jaunpur, U.P. for providing the laboratory facilities. The author is also thankful to National Botanical Research Institute (NBRI), Lucknow, U.P. for the verification & authentication of plant material (onion).

REFERENCES:

1. Anaissie EJ, Mc Ginnis RM, Paller MA, Clinical Mycology 1 st ed., New York: Churchill Livingstone, (2003).
2. Barrett D, From natural products to clinically useful antifungals. Biochim Biophys Acta, 1587: 224-233, (2002).
3. Benkeblia N, Antimicrobial activity of essential oil extracts of various onions (A. cepa) and garlic (Allium sativum). Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology, 37: 263-268, (2004).
4. Elnima EI, Ahmed SA, Mekkawi AG, Mossa JS, The antimicrobial activity of garlic and onion extracts. Pharmazie, 38(11): 747-758, (1983).
5. Fossen T, Pedersen AT, Andersen OM, Flavonoids from red onion (Allium cepa). Phytochemistry, 47: 281-285, (1998).
6. Gillespie SH, Evolution of drug resistance in Mycobacterium tuberculosis: clinical and molecular perspective. Antimicrobial Agents and Chemotherapy, 46: 267-274, (2002).
7. Graybill JR, The future of antifungal therapy. Clinical Infectious Diseases, 22: 166-177, (1996).
8. Grohs BM, Kunz B, Use of spice mixtures for the stabilisation of fresh portioned pork. Food Control, 11: 433-436, (2000).
9. Harris JC, Cottrell SL, Plummer S, Lloyd D, Antimicrobial properties of Allium sativum (garlic). Applied Microbiology and Biotechnology, 57: 282-286, (2001).
10. Hirvonen T, Virtamo J, Korhonen P, Albanes D, Pietinen P, Flavonol and flavone intake and the risk of cancer in male smokers (Finland). Cancer Causes and Control, 12: 789-796, (2001).
11. Ioku K, Aoyama Y, Tokuno A, Terao J, Nakatani N, Takei Y, Various cooking methods and the flavonoid content in onion. Journal of Nutritional Science and Vitaminology, 47: 78-83, (2001).
12. Kosmider B, Osiecka R, Flavonoid compounds: A review of anticancer properties and interactions with cisdiamminedichloroplatinum (II). Drug Development Research, 63, 200-211, (2004).
13. Lanzotti V, The analysis of onion and garlic. Journal of Chromatogrphy A 21: 1112(1-2): 3-22, (2006).
14. Navas PB, Carrasquero-Duran A, Flores I, Effect of black tea, garlic and onion on corn oil stability and fatty acid composition under accelerated oxidation. International Journal of Food Science and Technology, 41: 243-247, (2006).
15. Naz S, Siddiqi R, Asad Sayeed S, Effect of flavonoids on the oxidative stability of corn oil during deep frying. International Journal of Food Science and Technology, 43: 1850-1854, (2008).
16. Ramos FA, Takaishi Y, Shirotori M, Antibacterial and antioxidant activities of quercetin oxidation products from yellow onion (Allium cepa) skin. Journal of Agricultural and Food Chemistry, 54: 3551-3557, (2006).
17. Rauha JP, Remes S, Heinonen M, Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology, 56: 3-12, (2000).
18. Rose P, Whiteman M, Moore PK, Zhu YZ, Bioactive S-alk(en)yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. Natural Product Reports, 22: 351-368, (2005).
19. Shon MY, Choi SD, Kahng GG, Nam SH, Sung NJ, Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. Food and Chemical Toxicology, 42: 659-666, (2004).
20. Skerget M. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry, Barking, 89: p.191-198, (2005).
21. Sofia PK, Prasad R, Vijay VK, Srivastava AK, Evaluation of antibacterial activity of Indian spices against common foodborne pathogens. International Journal of Food Science and Technology, 42, 910-915, (2007).
22. Taguri, T., Tanaka, T. & Kouno, I. (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. Biological & Pharmaceutical Bulletin, 27, 1965-1969.
23. Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez JA, Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet. International Journal of Food Science and Technology, 43: 526-531, (2008).
24. Xu HX, Lee SF, Activity of plant flavonoids against antibiotic-resistant bacteria. Phytotherapy Research, 15: 39-43, (2001).
25. Yin MC, Cheng WS, Antioxidant activity of several Allium members. Journal of Agricultural and Food Chemistry, 46: 4097-4101, (1998).